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Von Willebrand Factor and Soluble E-Selectin in Hyperlipidaemia: Relationship to Lipids and Vascular Disease

Andrew D. Blann,[1]* Alison Davis,[2] J. Paul Miller,[3] and Charles N. McCollum[4]

[1]Thrombosis, Haemostasis and Vascular Biology Unit, University Department of Medicine, The City Hospital, Birmingham, B18 7QH, United Kingdom
[2]Department of Chemical Pathology, South Manchester University Hospital, Didsbury, Manchester M20 8LR, United Kingdom
[3]Department of Medicine, South Manchester University Hospital, Didsbury, Manchester M20 8LR, United Kingdom
[4]Department of Surgery, South Manchester University Hospital, Didsbury, Manchester M20 8LR, United Kingdom

Our objective was to determine whether endothelial cell products von Willebrand factor and soluble E-selectin are related to serum lipids, lipoprotein (a), and vascular disease in patients with hyperlipidaemia. In order to achieve our aim, blood samples were obtained for four experiments from 1) 160 patients (49 with symptomatic vascular disease) with hypercholesterolaemia and an equal number of age and sex matched controls; 2) 31 patients who were studied serially before and after successful resolution of their hypercholesterolaemia; 3) 15 patients with hypertriglyceridaemia; and 4) 20 controls, half of whom consumed a lipid-rich breakfast. von Willebrand factor and soluble E-selectin were measured by enzyme linked immunosorbent assay (ELISA) using commercial reagents. In experiment (1) von Willebrand factor was increased in the patients with hypercholesterolaemia (*P*=0.0077) and was higher still in patients with vascular disease (P<0.0001). Soluble E-selectin was not influenced by hypercholesterolaemia or vascular disease. The correlation of von Willebrand factor with total and LDL cholesterol (both *P*<0.001) remained after both age and blood pressure were controlled. Experiment (2) showed that serial studies in patients over an average of 7 months a reduction in total cholesterol was associated with a reduction in von Willebrand factor (r=0.51, *P*=0.002). Experiment (3) demonstrated that von Willebrand factor was not increased in patients with hypertriglyceridaemia (median 8.9 mmol/L), and in experiment (4) a lipid-rich breakfast taken by fasted, healthy controls produced an increase in serum triglycerides (*P*<0.01) but did not influence von Willebrand factor over an 8 hour period. We conclude that von Willebrand factor, but not soluble E-selectin, is raised in hypercholesterolaemia and therefore may be a potential indicator of endothelial cell physiology in subjects with, or at risk of, atherosclerotic vascular disease. Am. J. Hematol. 55:15–23, 1997. © 1997 Wiley-Liss, Inc.

Key words: von Willebrand factor; endothelial cells; cholesterol; triglycerides, Lp(a); soluble E-selectin; HDL-cholesterol; LDL-cholesterol; atherosclerosis

INTRODUCTION

Atherosclerosis, a major factor in coronary and peripheral artery disease, accounts for the majority of deaths in North America and Western Europe [1,2]. Considerable epidemiological evidence has established a direct relationship between increased total plasma cholesterol concentrations and the development of atherosclerosis. A similar link has been shown for raised concentrations of individual atherogenic lipoproteins, in particular the low density lipoprotein (LDL) fraction [3,4]. In contrast, a strong inverse relationship has been found between high density lipoprotein cholesterol (HDL-cholesterol) and the risk of coronary heart disease [5,6]. Although there is dispute as to the position of increased triglycerides as an independent risk factor [7], high concentrations are usually accompanied by low concentrations of HDL-cholesterol, and this may contribute to their link with risk

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*Correspondence: Dr. Andrew Blann, University Department of Medicine, The City Hospital, Dudley Road, Birmingham, B18 7QH, U.K.

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of coronary artery disease. More recently, angiographic studies suggest that the level of lipoprotein (a) [Lp(a)] is also a risk factor for both coronary artery and peripheral vascular disease [8,9].

The precise initiating events in the development of atherosclerosis are unclear but are believed to involve injury to the endothelium [1]. Increased circulating levels of von Willebrand factor (a specific product of the endothelium) are considered to be a marker of endothelial cell dysfunction. Levels of von Willebrand factor are raised in a variety of conditions where damage to the vasculature is common, for example in the inflammatory vasculitides [10,11].

Activation of endothelial cells by cytokines is accompanied by the appearance at the cell surface of increased numbers of leukocyte adhesion molecules, including E-selectin, another specific product of the endothelium. Soluble E-selectin can be found in tissue culture supernatants from activated endothelial cells and in the plasma and raised plasma levels have been reported in diabetes and hypertension. It has been suggested that increased levels of soluble E-selectin are also indicative of damage to the endothelium [13–15].

Since it is evident that hyperlipidaemia is linked with the development of atherosclerosis [3–6,16,17], this study tested the hypothesis that in patients with this risk factor there would be evidence of changes in endothelial integrity. Our first null hypothesis was that levels of soluble E-selectin and von Willebrand factor would be associated with those of total cholesterol and its subfractions, triglycerides and Lp(a). Our second hypothesis concerned the notion that changes in levels of lipoproteins in individuals with hypercholesterolaemia would be associated with alterations in both endothelial cell products. We tested our hypotheses in a prospective study, taking blood samples from non-diabetic patients attending hospital for dyslipidaemia, and from healthy controls.

There were four prospective experiments: (1) a large case control study of 160 patients (some of whom had concurrent symptomatic atherosclerosis) and matched healthy volunteers; (2) a serial study of 31 patients before and after successful drug or dietary treatment of their hypercholesterolaemia; (3) a small cross-sectional study of 15 patients with hypertriglyceridaemia; and (4) a brief serial study of 20 controls, half of whom consumed a lipid-rich breakfast and from whom serial blood samples were taken throughout the day.

MATERIALS AND METHODS

Subjects

The project had the approval of the Ethics Committee of South Manchester Health Authority and informed consent was obtained from all subjects. Patients with venous ulcers, gangrene, acute thrombotic events, diabetes, connective tissue diseases, erythrocyte sedimentation rate >20 mm in an hour, malignancy, or thyroid, renal, or liver disease were excluded as these can or may influence levels of von Willebrand factor [11]. Other risk factors for atherosclerosis [systolic and diastolic blood pressure (SBP, DBP, mm Hg), body mass index (BMI, kg/m^2), and current smoking] were recorded in all subjects as they are also independent influences on von Willebrand factor [18,19]. All blood samples (except in experiment 4) were taken between 9 a.m. and noon.

Experiment 1: The Case-Control Study

Cases were recruited from a dedicated Lipid Clinic. They included patients with familial hypercholesterolaemia (FH, defined as fasting serum cholesterol >7.5 mmol/L or LDL cholesterol >4.9 mmol/L with tendon xanthomata in the patient or a first degree relative [17]) and patients who failed to reach these criteria were classified as having non-familial hyperlipidaemia (non-FH). Patients with type III, type V hyperlipidaemia, or secondary hyperlipidaemia (e.g., to thyroid disease or obesity) were excluded from this case control study. Patients were also classified by the presence of symptomatic coronary artery disease (for example, previous percutaneous transluminal coronary angiography, myocardial infarction, or coronary artery bypass grafting), or peripheral vascular disease (such as Doppler-proven femoral artery, iliac artery, or carotid artery stenosis >75% or surgery to these arteries). All such vascular patients were in the stable phase of their disease with no case of acute thrombotic events or recent (>7 weeks) myocardial infarction or any surgery.

One hundred sixty controls were recruited from hospital staff and from patients without symptomatic vascular disease attending hospital for varicose veins, minor operations (e.g., removal of sebaceous cyst), hernia repair, or for endoscopy. They were gender and age-matched (within 2 years) to the cases. None of the controls were taking drugs designed to reduce levels of plasma lipids.

Experiment 2: Serial Studies on the Influence of Lipid Lowering

The influence of lipid lowering regime on changes to the lipid profile and von Willebrand factor was tested in 34 paired samples from 31 patients. Recruitment criteria were age at least 40 years with an initial cholesterol of >6 mmol/L and clinic visits at least 4 months apart. Patients were prescribed a full range of drugs designed to reduce lipids (mostly statins, fibrates, and resins) and some were treated with dietary advice alone.

Experiment 3: The Influence of Triglyceride-Rich Lipoproteins

von Willebrand factor was measured in 15 patients from the Lipid clinic. Recruitment criteria was a fasting triglyceride level >5 mmol/L. In these patients a variable degree of hypercholesterolaemia was also present. The experiment was controlled by both normal controls and by patients without hypertriglyceridaemia but with hypercholesterolaemia who were in the cross sectional study.

Experiment 4: A Lipid-Rich Breakfast Compared to a Normal Diet

The acute influence of alimentary lipaemia on levels of von Willebrand factor was examined by taking serial blood samples from ten healthy volunteers (five men, aged 32±6 years, no smokers), who, after an overnight fast, consumed 0.5 L double cream and two cream cakes at 9 a.m (approximately 100 g of fat). Blood was taken before this meal and at two hourly intervals throughout the day until 5 p.m. Subjects ate normally for the remainder of the day. This experiment was controlled by similar serial samples from ten age and sex matched subjects who had taken only their normal diet.

Laboratory Methods

All laboratory indices were estimated in fasting venous blood. Total cholesterol and triglycerides were measured using standard enzymatic techniques. HDL was measured following heparin-manganese precipitation. These analyses were performed by the Chemical Pathology Service of the University Hospital of South Manchester which participates in the UK National External Quality Assurance Scheme. LDL-cholesterol was calculated where possible by the Friedewald formula. Lp(a) was measured by a commercial ELISA kit (Immuno, Ltd., Arctic House, Rye Lane, Dunton Green, Sevenoaks, Kent, TH14 5HB, UK).

von Willebrand factor, which has a day to day variance of <10%, was measured by a standardised in-house ELISA technique by using polyclonal rabbit anti-human von Willebrand factor antisera (Dako, Ltd., 16 Manor Courtyard, Hughenden Avenue, High Wycombe, Bucks, UK). A WHO International Standard (National Institute for Biological Standards and Controls, Blanche Lane, South Mimms, Potters Bar, Herts, U.K.) was used throughout the study as previously described extensively by us elsewhere [18,19]. Cross-dilution experiments indicate that lipaemia does not interfere with the von Willebrand factor assay. Furthermore, von Willebrand factor is found in the HDL fraction prepared by ultracentrifugation, not in the LDL fraction (data not shown). Soluble E-selectin was measured by a commercial ELISA kit (R&D Systems, Ltd., 4-10 The Quadrant, Barton Lane, Abingdon, Oxon, UK), which has a sensitivity of <0.1 ng/mL. Briefly, 100 mL of a 1/20 dilution of serum is applied in duplicate to microtitre plates pre-coated with a monoclonal antibody to E-selectin. A second antibody-horse radish peroxidase conjugate, recognising a different epitope, is added in 100 μl aliquots at the same time and the plate is incubated for 90 min at room temperature. Following washes with 300 ml of buffer, 100 mL enzyme substrate is applied for approximately 10 min, the reaction terminated with 100 ml acid, and the optical density obtained from a plate reader at a wavelength of 450 nm. The assay is standardised with recombinant E-selectin supplied with the kit. For all ELISA's, interassay variance was <9%, intra-assay variance was <4%.

Statistics

A power calculation of the number of subjects required to provide a significant difference of 0.01 with a power of 0.95 was performed according to the method described by Altman [20] based on our previous experience with von Willebrand factor and soluble E-selectin [18,19,21,22]. Data distributed normally was analysed by unpooled student's t-test and analysis of variance and is presented as mean and standard deviation. Data distributed non-normally [triglycerides, Lp(a)] were analysed by the Mann-Whitney U test and Kruksal-Wallis test and is presented as median and range. Categorical data (gender, smoking habit, clinical diagnoses, and treatments) were analysed by the 2×2 chi-squared test. Serial data from patients treated with hypolipidaemic drugs/diet (experiment 2) were analysed using paired t-testing whilst serial data from the controls in the lipid breakfast (experiment 4) were analysed by repeated measures analysis of variance. In experiments 1 and 2, data were correlated according to Spearman's method and stepwise multivariate linear regression analysis with von Willebrand factor (both experiments) and soluble E-selectin (experiment 1 only) as dependent variables. In experiment 3 (hypertriglyceridaemia vs. hypercholesterolaemia versus controls) analysis of variance was used. All analyses were performed on a Minitab 8 extended package (Minitab, Inc., 3081 Enterprise Drive, State College, Philadelphia PA 16801).

RESULTS

Experiment 1: The Cross-Sectional Study

Results comparing the 111 patients with hyperlipidaemia but without clinical vascular disease with their age and sex matched controls are shown in Table I. Results for the 49 patients with hyperlipidaemia and symptomatic vascular disease versus their controls are shown in Table II. In both sets of analyses, SBP, cholesterol and LDL-cholesterol, von Willebrand factor, and Lp(a) were higher in the patient groups whilst HDL-cholesterol was

TABLE I. Demographic, Risk Factor, and Endothelial Cell Markers in Patients With Hyperlipidaemia and Their Controls

Index (unit)	Patients	Controls[a]	
n	111	111	*P* value[a]
Systolic blood pressure (mm Hg)	137 ± 23	130 ± 17	$P = 0.014$
Diastolic blood pressure (mm Hg)	83 ± 15	78 ± 12	$P = 0.0082$
Cholesterol (mmol/L)	7.6 ± 1.6	5.5 ± 1.2	$P < 0.0001$
Triglycerides (mmol/L)	1.5 (0.4–7.7)	1.5 (0.5–4.0)	n.s.
HDL (mmol/L)	1.3 ± 0.4	1.5 ± 0.4	$P = 0.0048$
LDL (mmol/L)	5.5 ± 1.6	3.4 ± 1.0	$P < 0.0001$
Lp (a) (ng/ml)	17.0 (0–97.0)	12.8 (0–57.5)	$P = 0.0032$
Smokers (number)	24	28	n.s.
Age (years)	48 ± 12	49 ± 11	n.s.
Men (number)	50	50	n.s.
BMI(kg/m^2)	25.8 ± 4.4	25.8 ± 4.5	n.s.
von Willebrand factor (kIU/L)	1.17 ± 0.39	1.03 ± 0.37	$P = 0.0077$
Soluble E-selectin (ng/mL)	54 ± 19	53 ± 19	n.s.

[a]n.s., not significant. See text for abbreviations.

TABLE II. Demographic, Risk Factor, and Endothelial Cell Markers in Patients With Hyperlipidaemia and Symptomatic Vascular Disease, and Their Controls

Index (unit)	Patients	Controls[a]	
n	49	49	*P* value[a]
Systolic blood pressure (mm Hg)	141 ± 21	131 ± 18	$P = 0.017$
Diastolic blood pressure (mm Hg)	84 ± 13	79 ± 11	n.s.
Cholesterol (mmol/L)	7.3 ± 1.7	5.4 ± 1.2	$P < 0.0001$
Triglycerides (mmol/L)	1.5 (0.6–3.8)	1.5 (0.5–6.7)	n.s.
HDL (mmol/L)	1.3 ± 0.4	1.5 ± 0.4	$P = 0.0008$
LDL (mmol/L)	5.3 ± 1.7	3.1 ± 1.2	$P < 0.0001$
Lp (a) (ng/ml)	24.0 (0–93.0)	11.7 (0–80.0)	$P = 0.0026$
Smokers (number)	9	9	n.s.
Age (years)	53 ± 10	54 ± 8	n.s.
Men (number)	40	40	n.s.
BMI (kg/m^2)	25.3 ± 2.9	24.3 ± 2.6	n.s.
von Willebrand factor (kIU/L)	1.29 ± 0.36	0.93 ± 0.30	$P < 0.0001$
Soluble E-selectin (ng/mL)	54 ± 32	54 ± 16	n.s.

[a]n.s. = not significant. See text for abbreviations.

lower. There were no differences in triglycerides, proportion of smokers, BMI or soluble E-selectin. There were no differences in age or the proportions of the sexes as these were recruitment criteria for the controls. DBP in the patients was significantly higher than that of their controls only in the group without symptomatic vascular disease (Table I).

Comparing the two groups of patients, there was no difference in SBP or DBP, cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, Lp(a), proportion of smokers, BMI, percentage of men (45% in the group

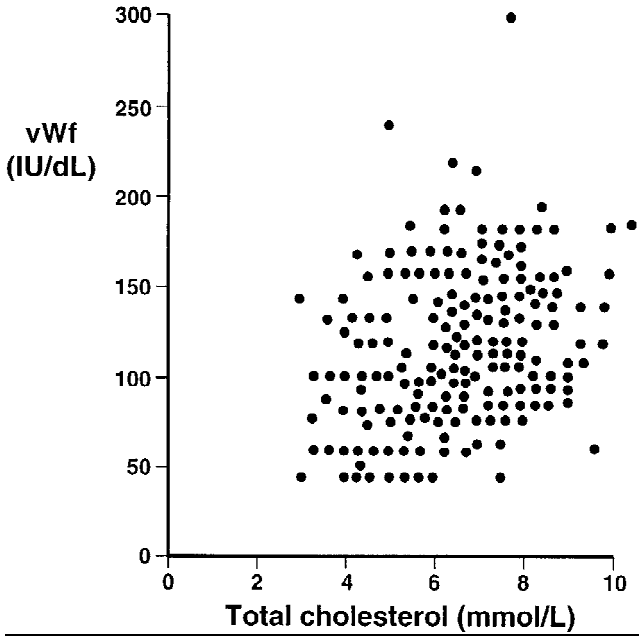

Fig. 1. Correlation between von Willebrand factor and total cholesterol (r=0.25, $P<0.001$).

without vascular disease, 61% in the group with vascular disease, $\chi^2 = 3.699$), or soluble E-selectin. The patients with symptomatic vascular disease were older (53±10 years vs. 48±12, $P = 0.0023$) and had higher levels of von Willebrand factor (vWf) (1.29±0.36 kIU/L vs. 1.17±0.39, $P = 0.047$) than asymptomatic patients.

An analysis of the patients according to the background of their hypercholesterolaemia failed to reveal any differences in any of the indices we recorded. There were no differences in the proportions of patients with FH or non-FH in the two groups of patients regarding symptomatic vascular disease (present in 26% of patients with FH vs. 33% of patients with non-FH [$\chi^2 = 0.888$]).

Correlations. Stepwise multivariate linear regression analysis of the data from all subjects revealed significant independent relationships between von Willebrand factor and total cholesterol ($r = 0.25$, $P<0.001$, Fig. 1), LDL-cholesterol ($r = 0.23$, $P<0.001$), SBP, ($r = 0.27$, $P = 0.007$), and age ($r = 0.22$, $P = 0.009$). There were no correlations between von Willebrand factor and Lp(a) in any group (controls and/or patients). There were weakly significant univariate relationships between soluble E-selectin and both sex (higher in men, $r = 0.15$, $P = 0.043$) and body mass index ($r = 0.15$, $P = 0.045$). However, there were no significant multivariate relationships between soluble E-selectin and any of the other variables measured. The essentially negative findings regarding the relationship between soluble E-selectin and hypercholesterolaemia prompted us to refrain from measuring the former molecule in the serial and triglyceride experiments which follow. Within the group of 160 patients alone, there were univariate von Willebrand factor cor-

TABLE III. Serial Studies in Patients With Hypercholesterolaemia Before and After Treatment*

	1st clinic visit	2nd clinic visit	*P* value
Index			
Cholesterol (mmol/L)	7.6 ± 1.0	6.7 ± 1.1	0.0001
Triglycerides (mmol/L)	1.6 (0.4–4.3)	1.5 (0.6–4.3)	n.s.
HDL (mmol/L)	1.3 ± 0.3	1.5 ± 0.3	0.0014
LDL (mmol/L)	5.5 ± 1.0	4.8 ± 1.2	0.0001
von Willebrand factor (kIU/L)	1.58 ± 0.44	1.30 ± 0.38	0.0009

*Data are mean and standard deviation or median and range.

relations with both total cholesterol ($r = 0.18$, $P = 0.032$) and SBP ($r = 0.16$, $P = 0.041$) although only the former remained in multivariate analysis.

Anti-hyperlipidaemia treatment. Analysis of the treatment regimes of the patients revealed that 31% were not on active drug treatment and this reflects the proportion of patients who were new referrals or were in initial analysis of their hypercholesterolaemia. The most frequently prescribed agents were resins alone (taken by 29% of the patients being treated with drugs), statins alone (28%), and the combination of a resin and a statin (25%). Significantly more patients without symptomatic vascular disease were being treated with resins than those with vascular disease (21% vs. 12.5%, $P<0.05$) and more patients with vascular disease were being treated with statins than those free of vascular disease (27% versus 9%, $P<0.05$). Thirty-eight percent of patients free of vascular disease were not being treated with active drug therapy (i.e., by diet alone) but only 19% of patients with symptomatic vascular disease were not on an active drug ($P<0.05$).

Experiment 2: Serial Studies of Patients Being Treated for Hypercholesterolaemia

In the group of 31 patients as a whole, there were overall reductions in von Willebrand factor, total cholesterol, and LDL-cholesterol, while HDL-cholesterol increased in 34 paired samples taken at clinic visits a median of 7 months (range 4–12 months) apart (Table III). The reduction in von Willebrand factor (from a mean of 1.58 kIU/dL to a mean of 1.30 kIU/dL) is greater (i.e., 18%) than can be accounted for by day to day variation in the assay for von Willebrand factor (±10%). We cannot account for the apparently high levels in this mixed subgroup of 31 patients, whose recruitment was based on hypercholesterolaemia alone, and assume it must be due to random distribution. Levels of triglycerides were unchanged. In multivariate analysis the change in von Willebrand factor correlated only with the change in total cholesterol ($r = 0.51$, $P = 0.002$, Fig. 2), not the changes in LDL-cholesterol ($r = 0.39$, $P = 0.099$) or HDL-cholesterol ($r = 0.01$, $P = 0.937$).

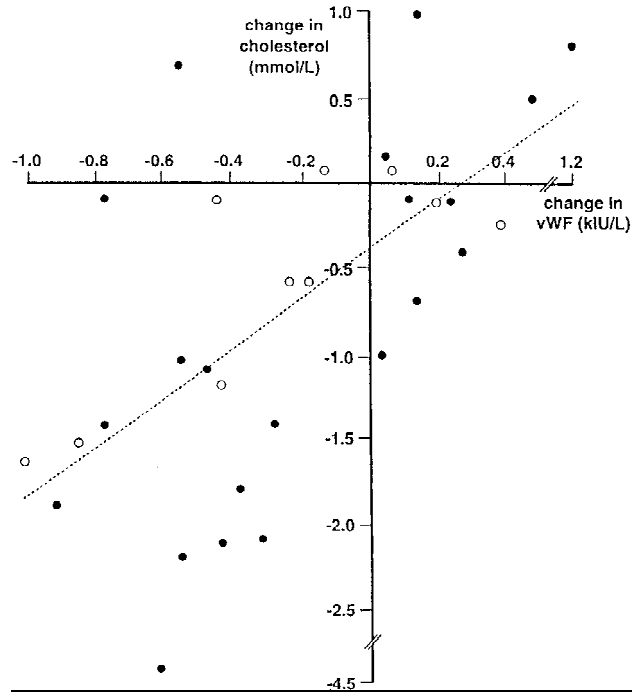

Fig. 2. The relationship between change in von Willebrand factor and change in total cholesterol in the serial study. Open circles are patients without symptomatic vascular disease, closed circles are patients with established vascular disease.

Similar changes were also seen in the subgroup of 18 patients without symptomatic vascular disease. von Willebrand factor fell from 1.53±0.54 kIU/L to 1.22±0.41 ($P = 0.015$), total cholesterol from 7.4±0.9 mmol/L to 6.7±1.0 ($P = 0.0011$), LDL from 5.3±0.9 mmol/L to 4.4±1.0 ($P = 0.0005$), while HDL increased from 1.3±0.35 mmol/L to 1.6±0.3 ($P = 0.0028$). Levels of triglycerides were unchanged. In multivariate analysis the change in von Willebrand factor correlated with the change in total cholesterol ($r = 0.58$, $P = 0.009$), not the change in LDL-cholesterol ($r = 0.38$, $P = 0.148$) or the change in HDL-cholesterol ($r = 0.048$, $P = 0.859$). The pattern was again similar in the subgroup of 13 patients with symptomatic vascular disease over the same time period with falls in von Willebrand factor (1.64±0.26 kIU/L to 1.4±0.33, $P = 0.027$), cholesterol (7.8±1.1 mmol/L to 6.8±1.3, $P = 0.014$) and LDL (5.7±1.1 mmol/L to 4.8±1.3 mmol/L, $P = 0.013$) but there were no significant changes in triglycerides or HDL. On multivariate analysis none of these changes were significantly correlated.

Although there was a significant relationship between the fall in serum cholesterol and the fall in von Willebrand factor concentration our study lacked sufficient power to determine if the class of lipid lowering agent used to lower cholesterol influenced this relationship independently of its effect on serum lipids. Overall, 14

TABLE IV. von Willebrand factor and Lipid Indices in Patients With Moderate to Severe Hypertriglyceridaemia or Hypercholesterolaemia, and Controls*

	Chol. mmol/L	Trigs. mmol/L	HDL mmol/L	LDL mmol/L	vWf kIU/L
Normocholesterolaemic controls	5.3 ± 1.1	1.3 (0.6–3.8)	1.5 ± 0.3	3.2 ± 0.9	1.01 ± 0.34
Patients with hypertriglyceridaemia	8.9 ± 3.6	7.8 (5.2–43.5)	1.0 ± 0.2	–	0.98 ± 0.26
	$P = 0.003$	$P < 0.0001$	$P = 0.026$		$P = 0.878$
Hypercholesterolaemic controls	7.4 ± 1.5	1.5 (0.5–4.8)	1.3 ± 0.4	5.2 ± 1.4	1.20 ± 0.36
	$P = 0.009$	$p = 0.246$	$P = 0.046$	$P = 0.005$	$P = 0.013$

*Data are mean and standard deviation or median and range. Statistics are changes relative to the normal controls. vWf, von Willebrand factor. LDL data is missing as it cannot be calculated from the Friedewald formula in hypertriglyceridaemia.

patients were on diet alone, 12 were taking resins, 5 were prescribed statins, and 7 were on fibrates. Seven patients were taking more than one treatment. The degree of change of any of the indices was unrelated to the interval between visits.

Experiment 3: Influence of Triglyceride-Rich Lipoproteins on von Willebrand Factor

Blood samples were obtained from 15 patients (who were not in the case-control study) without symptomatic vascular disease whose serum triglycerides were >5 mmol/L. These patients were controlled by 15 patients with hypercholesterolaemia (matched for age and sex) and 15 normocholesterolaemic controls (matched for age and sex), who were selected from the subjects within the cross sectional study. Patients with hypertriglyceridaemia had levels of von Willebrand factor similar to those of the normocholesterolaemic controls but significantly lower than those of the hypercholesterolaemic controls. The serum cholesterol concentration in the two hyperlipidaemic groups were not significantly different (Table IV).

Experiment 4: A Lipid-Rich Breakfast Compared to a Normal Diet

The results of the alimentary lipaemia study are shown in Table V. A large peak in serum triglycerides and a more modest rise in cholesterol was seen at 1 p.m. in those taking the lipid-rich breakfast but no changes were seen in the controls eating their normal diet. No significant changes were seen in von Willebrand factor in either group.

DISCUSSION

The present case-control study (experiment 1) demonstrated increased von Willebrand factor in patients attending a Lipid Clinic, with further rises in those with symptomatic vascular disease. The increase in von Willebrand factor correlated independently with levels of total and LDL-cholesterol, SBP, and age. In the patients alone von Willebrand factor correlated with total cholesterol. Serial studies in a subgroup of these patients (experiment 2) indicated that reductions in cholesterol were associated with reductions in von Willebrand factor. Interestingly, both indices increased in five patients, and the overall relationship remained if these five were excluded from the analysis. However, this association may not be directly causal as we did not measure blood pressure before and after treatment. It may be that reduced levels of von Willebrand factor are due to reduced haemodynamic stress on the vasculature following falls in blood pressure, as we have shown that increased von Willebrand factor in hypertension falls if that hypertension is treated [23]. Curiously, in the triglyceride study (experiment 3, Table IV), patients with concurrent hypertriglyceridaemia and hypercholesterolaemia had normal levels of von Willebrand factor. An implication of this finding is that this combined dyslipidaemia does not damage the endothelium, and that hypertriglyceridaemia is, in this context, protective against the presumed cytotoxic effect of cholesterol and LDL-cholesterol (some of which may be oxidised). We are unable to offer a convincing alternative hypothesis for this perplexing finding. No increase in von Willebrand factor was seen during the acute hypertriglyceridaemia in alimentary lipaemia in normal subjects (experiment 4), even though there was a modest increase in cholesterol. This may be related to the cross-sectional triglyceride finding in the patients, although it should be recalled that the patients were older and were also suffering additional problems such as low HDL-cholesterol. Perhaps the younger controls (none of whom were smokers) in the diet experiment have better antioxidant protection against oxidised-LDL, thereby minimising the cytotoxic effect of this lipoprotein on the endothelium. These findings, together with the cross-sectional and serial data, lead us to suggest that triglyceride-rich lipoproteins do not influence levels of von Willebrand factor, though the possibility that a subgroup of particles, for example remnant lipoproteins, do influence von Willebrand factor cannot be excluded. Patients with type III dyslipidaemia were excluded from the case-control study.

In contrast to the von Willebrand factor data, we were

TABLE V. Post-Prandial Lipoproteins and von Willebrand factor in Controls Following a Lipid-Rich Breakfast

	Blood sample (time)				
	1 (9 a.m.)	2 (11 a.m.)	3 (1 p.m.)	4 (3 p.m.)	5 (5 p.m.)
Total cholesterol (mmol/L)					
Normal diet	4.6 ± 0.5	4.5 ± 0.5	4.6 ± 0.5	4.6 ± 0.5	4.6 ± 0.5
Lipid meal	4.9 ± 0.9	5.0 ± 0.9	5.2 ± 0.9*	4.9 ± 0.8	4.9 ± 0.9
Triglycerides (mmol/L)					
Normal diet	1.2 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	1.2 ± 0.4	1.2 ± 0.4
Lipid meal	1.1 ± 0.6	1.7 ± 0.7**	2.8 ± 1.7**	2.6 ± 1.8**	2.0 ± 1.5*
von Willebrand factor (kIU/L)					
Normal diet	0.93 ± 0.17	0.92 ± 0.15	0.91 ± 0.18	0.88 ± 0.17	0.89 ± 0.21
Lipid meal	0.91 ± 0.24	0.88 ± 0.22	0.88 ± 0.24	0.82 ± 0.28	0.91 ± 0.33

*, $P < 0.05$, **, $P < 0.01$ relative to the initial fasting sample taken at 9 a.m. Data are mean and standard deviation.

unable to find evidence of any change in levels of soluble E-selectin in hyperlipidaemic patients with or without overt vascular disease. Lp(a) was raised in hyperlipidaemic patients with and without symptomatic vascular disease compared with controls but was not significantly higher in those with symptomatic vascular disease. This is counter to some reports [8,24,25] but is concordant with others [26,27]. We are also unable to confirm reports of the inverse relationship between Lp(a) and triglycerides, or the positive relationship between Lp(a), total cholesterol, and LDL-cholesterol [9,28]. These discrepancies may be due to different patients studied and different techniques used to quantify Lp(a). We also note that levels of Lp(a) failed to correlate with those of von Willebrand factor.

Analysis of the data with respect to the class or dosage of drug therapy prescribed failed to provide any support for the hypothesis that the changes in von Willebrand factor we have found were due directly to drug action on the endothelium, though the study was not specifically designed to test this. Clinical decision to treat with any particular drug was not influenced and we were keen to avoid an effective drug trial. Consequently, the drug prescribing was very heterogeneous and difficult to interpret with sufficient clarity to warrant publication. However, adherence to a lipid-lowering diet without active drug support over a 3 year period is sufficient to reduce total and LDL cholesterol, triglycerides, and von Willebrand factor in patients with hypercholesterolaemia and ischaemic heart disease [29].

The precise pathogenesis of atherosclerosis has yet to be elucidated, reflecting its complexity, but injury to the endothelium could be an initiating and propagating event and high levels of certain lipoproteins could be a mechanism or a marker for this injury [1–9]. Although von Willebrand factor is found in platelets and megakaryocytes, expert opinion suggests that the two pools are non-interchangeable and that the contribution to the plasma pool from platelets is barely significant [10,30]. The other major risk factors for atherosclerosis (hypertension, obesity, smoking, diabetes, and hyperhomocysteinaemia) are associated with increased levels of von Willebrand factor [18,19,31,32]. The failure of von Willebrand factor to correlate with soluble E-selectin suggests that the latter may not be an index of endothelial cell dysfunction as proposed by others [15,16]. We were also unable to find increased soluble E-selectin levels in a separate study of patients with ischaemic heart disease or peripheral vascular disease [21]. Regarding the other major risk factors for atherosclerosis, we have found raised soluble E-selectin in patients with essential hypertension [22] and in non-insulin dependent diabetes mellitus [31]. It is therefore notable that we have not been able to find increased soluble E-selectin in hypercholesterolaemia.

Our finding of increased von Willebrand factor in hyperlipidaemic patients with symptomatic vascular disease when compared to patients without symptoms supports the hypothesis that atherosclerosis is related to vascular injury [1,2] and also the work of others. Smith et al. [33] and Cortellaro et al. [34] have reported increased von Willebrand factor in epidemiological studies of cardiovascular disease although neither examined the relationship with serum lipids. However, Conlan et al., in almost 12,000 community-recruited subjects [35], found an inverse relationship between von Willebrand factor and HDL-cholesterol and a positive correlation between von Willebrand factor and triglycerides, but none between LDL-cholesterol and von Willebrand factor, in contrast to our findings. These differences may be due to the nature of our two studies: ours set out to precisely test a hypothesis in an equal number of patients with hypercholesterolaemia and healthy subjects who were normocholesterolaemic. Consequently we had a relatively large cohort of subjects (i.e., 50% of all subjects) with established hypercholesterolaemia whereas the population study of Conlan et al. may not have had a sufficiently

large number/proportion of subjects with a sufficiently excessive hypercholesterolaemia to accurately test this hypothesis. Furthermore Conlan's population included diabetics and is very likely to include those with other risk factors for increased von Willebrand factor (e.g., rheumatoid arthritis [10,11]), which would confound a clear relationship and whom we excluded. However, close examination of their data reveals a very weak positive relationship between LDL and von Willebrand factor, and we both found correlations between von Willebrand factor and age, but none between von Willebrand factor and Lp(a). Wada et al. [36] failed to find a clear relationship between von Willebrand factor and hyperlipidaemia (including Lp(a)), in a study of 51 patients with ($n = 13$) or without ($n = 38$) concurrent ischaemic heart disease, and only 20 controls unmatched for age or gender. The reasons for the discrepancies between this study and our own are uncertain but ours included almost three times as many patients and eight times as many controls, and was matched for age and sex.

The mechanism for the increased von Willebrand factor in hypercholesterolaemic subjects in our study is unclear but may relate at least in part to levels of oxidised LDL as these are believed to be important in atherogenesis [37,38]. Both LDL and oxidised LDL are cytotoxic to endothelial cells in vitro and induce increased levels of von Willebrand factor in tissue culture supernatants [39–41]. Our present finding that von Willebrand factor correlates with systolic blood pressure confirms our previous work [18], the mechanism for which may relate to haemodynamic stress [42]. These two risk factors may be linked as it has been suggested that oxidised LDL leads to endothelial dysfunction, with alteration in endothelial-dependent relaxation, which may contribute to hypertension via reduction in EDRF/nitric oxide activity [43,44]. Therefore, from the strictly scientific viewpoint, it is conceivable that the cause of increased von Willebrand factor in our patients is hypertension, not hypercholesterolaemia, although we believe lipids to be responsible. Our present serological study supports invasive studies of the coronary circulation where successful cholesterol-lowering treatment was associated with an improvement in endothelial cell function assessed by angiography-detected response to acetylcholine [45,46].

We are unable to link underlying cause of the hypercholesterolaemia (i.e., FH or non-FH) with any measurement we recorded, suggesting that it is the hypercholesterolaemia itself, which is the important common pathway. von Willebrand factor is being increasingly regarded as a marker of endothelial cell injury, and our in vivo data support the hypothesis [1] that hypercholesterolaemia damages the endothelium, one of several potential mechanisms by which it may contribute to the atherosclerotic process. Increased levels of von Willebrand factor are a poor prognostic indicator in patients with hypertension [23], angina [47], and following a myocardial infarction [48]. Our data points to a further mechanism for this relationship and provides impetus for studies aimed at reducing levels of this procoagulant by inhibitory peptides or engineered heparins [49–51].

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